ROOT ROTS of Alfalfa and Red Clover

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Contents

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Introduction

IN THE United States and especially in the Appalachian region occupied by West Virginia, forage legumes are essential to the agricultural system. They provide a highly nutritive component of animal feeds, and are increasing in importance because of the emphasis on livestock production in the area. Red clover and alfalfa have long provided the bulk of legume forage cut as hay. White clover, including the Ladino selection, is of great value but primarily as a pasture legume. Certain other forage legumes add to our reservoir of nitrogen-fixing, nitrogen-conserving plants but to a lesser degree.

Neither red clover nor alfalfa is native to the Americas. Red clover was introduced at an early date during colonial times and became naturalized very readily. Its adaptability to a variety of environments in many areas of the world seems to indicate that the plant is relatively free of major disease problems, *but this is not true*. In most places where it is grown, it is considered a short-lived perennial, even a biennial. Only in a few locations are large numbers of individual vigorous plants able to survive for several years (6).

Alfalfa was introduced during colonial times but was less widely grown than red clover. It has less tendency to spread as a naturalized plant in most environments but is more vigorous and historically appears to be less damaged by diseases than is red clover. An overall look at these two legumes reveals that alfalfa is a long-lived perennial and red clover a short-lived one.

It is unknown when these legumes were introduced into what is now West Virginia. It is known that red clover was grown in Rhode Island in 1663 and that quantities of seed were imported from England in later years (35). Seed was carried across the country by the earliest farmers and clover was incorporated into their cropping systems.

Alfalfa also was introduced to the East Coast at an early date. Tysdal and Westover (41) record that both George Washington and Thomas Jefferson had fields of alfalfa (lucerne) in the early 1790s. Alfalfa was

grown with "some" success during these times in Virginia, North Carolina, Pennsylvania and New York. Most of the alfalfa in this country, however, was derived from seed brought from Chile to California by gold prospectors that were making the trip around the Horn (41). Its introduction into West Virginia was likely at a relatively late date.

The agricultural census report for 1900 (42) lists 123 acres of alfalfa and 25,170 acres of clover grown in West Virginia in 1899. Red clover probably made up the bulk of this clover acreage. It seems that growers had little success with alfalfa until its requirements were better understood. Success depended largely on proper cultural practices and as the farmers were informed of its requirements, the use of this crop increased rapidly. Alfalfa acreage in West Virginia was 164,000 by 1959 (30) compared with 362,000 acres in clover, timothy and mixtures.

Maintenance of Stand Problems

The literature on the culture of alfalfa and red clover and growers indicate that obtaining a stand and maintenance of that stand is a constant problem.

The purpose of this publication is to review the general problems of these crops as they relate to diseases, particularly root and crown rots.

Numerous factors adversely affect the growth of these legumes. These are best summarized in a textbook on forage management by Smith (38).

Following is a list of problems in red clover culture. In some instances one problem is intimately associated with another, for example, numbers 5, 6, and 7.

1. Highly variable species with strains from one region having different characteristics from strains originating in other areas.
2. Intolerant of acid soils.
3. Requires high levels of all basic nutrients (except nitrogen) from the soil.
4. Low tolerance to extreme cold and generally less cold hardy than alfalfa.
5. Adversely affected by high temperatures.
6. Adversely affected by low moisture conditions.
7. Does not flourish on light soils.
8. Does not tolerate excessively wet soils but will grow on wetter soils than alfalfa.
9. Subject to frost heaving in areas of repeated freezing and thawing.
10. Will not tolerate close grazing.

11. Numerous damaging diseases and insect pests widely distributed in the areas where red clover is grown.

Problems inherent in alfalfa culture are similar but certain differences can be noted:

1. Very intolerant to acid soils.
2. Needs soil with good surface drainage and/or subsurface drainage.
3. Will not tolerate wet soil.
4. Needs high levels of basic nutrients except nitrogen in soil.
5. Will not tolerate excess cold. Numerous varieties are not winter hardy but generally more tolerant than red clover.
6. Subject to frost heaving, particularly in heavier soils.
7. Does not flourish in areas of reduced solar radiation.
8. Sometimes needs inoculation with suitable *Rhizobium* species.
9. Many damaging diseases and insect pests widely distributed in the areas where alfalfa is grown.

In some of the earlier forage legume studies, several writers (12, 15, 44, 48) pointed out the probably relationship between those conditions that adversely affect the plant and the frequency or severity of disease occurrence. Until about 1920, most failures of alfalfa and red clover were attributed directly to one or more of these factors other than disease. In recent years, relationships have been repeatedly found proving the intimate association between disease and the other adverse factors that may affct the plant's vigor. A plant may be weakened by one or more unfavorable factors, thus permitting disease organisms to invade the tissues and kill it. Cause of the plant's death may be difficult to pinpoint, one may say it was caused by the imbalance of nutrients or another that it was cold injury or still another may say it was caused by a pathogenic organism.

Red Clover Diseases

Root-rot is clearly a greater problem in red clover than in alfalfa. The average annual loss of red clover for the years 1951-1960 attributed to crown and root rots was 23 per cent, compared to only 2 per cent for alfalfa (43). These percentages seem low for West Virginia, particularly for alfalfa. A 5 per cent loss would be more in line with our observations.

Sheldon (37), near the turn of the century, stated that farmers had considerable difficulty in obtaining permanent stands of clover in the Ohio and Kanawha valleys. He concluded after a limited investigation that the problems were largely of a cultural nature and there was nothing

to indicate a fungus disease was involved. Sheldon's conclusion may have been correct but the root-rot situation at the present time would lead forage pathologists to question the report.

SCLEROTINIA ROOT AND CROWN ROT

One of the diseases involving the crown and roots of red clover is caused by the fungus *Sclerotinia trifoliorum* Erikss. It was recognized in Germany as early as 1857 (36), and by 1897 (9) had been established as a cause of clover sickness in England. Millspaugh (31) recorded a collection of this fungus in West Virginia made by Nuttall in 1893, but there were no other records of it in the region until much later.

Nine states reported the disease by 1919—Delaware, Kentucky, New York, New Jersey, Virginia, Indiana, Oregon, North Carolina and South Carolina (47).

Archer (3) did not include *Sclerotinia* in his list of alfalfa and clover diseases of West Virginia published in 1929. He stated, however, that "fields in the mountainous parts of the state are severely affected by crown rot but fields in the Eastern Panhandle are practically free of the trouble." Perhaps he was referring to this disease.

Atkinson, Kreitlow and Leach (4) reported that *Sclerotinia* appeared on several legumes including red clover and alfalfa in 1945 but they made no estimate of the losses from this fungus. A statement made in 1952 (14) on the severity of *Sclerotinia* in red clover may have been the first indication that it was of major importance in West Virginia, but circumstantial evidence indicates that it was a cause of major losses many years prior to that date. It is probable that the fungus was not recognized by the individuals working with the crops or was not evident at the time that observations were made.

At the present time *Sclerotinia* crown rot can be found regularly during the winter in red clover fields but it is most evident during early spring just as new growth is starting. At this time infected plants quickly wilt and die. The white fluffy mycelial growth of the fungus is often evident on the crown of the diseased plant and sometimes on the surrounding soil. The best means of identifying the fungus is to find the black, irregularly shaped sclerotia, sometimes as large as pencil erasers, that develop in the decaying tissues. Infected plants seldom recover, but warm and dry periods may check growth of the fungus. Partially decayed plants may survive for some time but usually die rapidly from other causes during the summer months.

Losses from *Sclerotinia* are not difficult to assess but there may be considerable variation from field to field. Losses of 25 per cent are not uncommon and at times losses may be considerably greater. An overall

estimate of losses over a five-year-period would be about 5 per cent because of year-to-year variation in intensity of the disease.

FUSARIUM ROOT ROTS

The earliest report of *Fusarium* being associated with clover is by Young (48), who concluded that certain species of *Fusarium* isolated from clover roots were weakly parasitic but required the assistance of other deleterious agents to kill the plants.

Fergus and Valleau (15) investigated the cause of red clover failure in Kentucky and arrived at conclusions similar to those generally agreed upon at the present time. They found that several *Fusarium* species were present in the rotting roots and that they were pathogenic because of their ability to induce severe injury to clover and alfalfa seedlings under laboratory conditions. Their conclusions concerning the relationship of unfavorable environmental conditions to clover failure should be noted here. From field observations they reported that clover failure results indirectly from an unfavorable environment and more directly from the result of attack by pathogenic organisms upon the roots. They listed a number of factors as contributing to the unfavorable environment; these included unfavorable plant nutrition, high temperatures and drought. They also noted that root rot is universal in regions where the red clover plant has been long cultivated and that it becomes a critical factor only when the environmental conditions are unfavorable to continuous growth of the plant.

Consequently it would appear that the problem was solved many years ago and accumulating evidence supports the conclusions reached by Fergus and Valleau. But not all agricultural scientists agree that the problem of clover failure is so intimately related to invasion by pathogenic fungi. In looking for the causes of the problem they have placed more emphasis on the nutritional, environmental, and other factors that adversely affect the plant with a guarded admission that microorganisms do eventually decay the plant and may even kill it. Perhaps the greatest fallacy to this reasoning was their failure to recognize that when the decay is not present, plants have a much greater capacity and opportunity to recover once drought conditions are overcome, mineral nutrients replaced, or similar problems corrected.

Only in recent years have forage scientists recognized the magnitude of the root-rot problem. This has led to a regional effort by workers in agricultural experiment stations in several of the northeastern states to investigate legume root rots more critically to find better control measures. The opportunity to improve these crops is apparent (43) in the 23 per cent loss estimated for red clover and a smaller but still significant 10 per

cent root-rot loss in white clover (including ladino). Only a 2 per cent overall loss is estimated for alfalfa but it is much greater in some varieties and in some locations.

Several different symptoms are associated with the attack of various *Fusarium* species present but specific types of injury usually can not be connected with any single *Fusarium* species. Lesions are frequently confined to the cortex (outer layer) of the tap root or lateral roots and may or may not be associated with visible wounds on the root surface. In other instances discoloration is largely restricted to the central core and may follow the vascular tissues in irregular patterns. Both cortex and central core are usually invaded as the disease progresses. As the functioning root tissues are killed, wilting of the leaves occurs and the plant dies but often only after a prolonged period of declining vigor. Root lesions and internal discolorations may be any of several shades of brown. It is not uncommon for the lower parts of the root to be completely decayed and for several lateral roots from near the crown to replace the tap root but these then decay in turn. The necrotic areas that appear in the pith of the crown described by Graham *et al.* (20) as internal breakdown might be confused with damage by pathogenic organisms. (see Plate I, illustration 1). Internal breakdown is classified as a physiogenic disease but it may be found in plants also damaged by *Fusarium* species.

FUNGI ISOLATED FROM ROTTING RED CLOVER ROOTS

The predominant species of fungi associated with red clover roots can be determined by conventional isolation techniques. In order to find what fungi, particularly potential pathogens, are in the northern West Virginia area, samples were taken from 27 different clover fields in several counties. Five samples of 2-year-old plants were taken from favorable sites in fields or those areas where plant growth was greatest and the highest population survived. Five additional sample plants were taken from unfavorable sites where the population of plants was low or plants were less vigorous. Crown and tap root sections were surface sterilized, split open, and small pieces removed and plated on glucose-yeast extract (10g — 2g) agar in petri dishes. Sections of lesions on root surfaces and small sections of lateral roots were also cut, surface sterilized and plated. The total numbers of isolates obtained from 270 plants are shown in Table 1. These were identified to genus except for *Fusarium* and a few other genera. Nineteen other genera were isolated once or twice but are not listed in Table 1 because of their infrequent occurrence.

Several fungi commonly associated with the soil and usually classified as saprophytes are included in this list. These may have invaded decayed root tissues or may not have been removed from the surface by the

TABLE 1
Fungi Isolated from 270 Two-Year-Old Red Clover Roots Growing In Both Favorable and Unfavorable Areas

	Number of Isolates
Fusarium oxysporum	305
Fusarium roseum	245
Fusarium solani	168
Trichoderma viride	135
Gliocladium roseum	111
Rhizoctonia sp.	62
Phoma sp.	57
Fusarium moniliforme	48
Cephalisporium sp.	19
Penicillium spp.	19
Rhizopus sp.	17
Aspergillus spp.	14
Mucor sp.	14
Mycogone sp.	12
Coniothyrium sp.	7
Alternaria sp.	7
Zygorhynchus sp.	6
Chaetomium sp.	4
Diplodia sp.	3
Sepedonium sp.	3
Leptodiscus sp.	2

sterilization process. On the basis of known association with other diseases, *Fusarium oxysporum, F. roseum, F. moniliforme, F. solani, Gliocladium roseum, Rhizoctonia* sp., *Phoma* sp., and *Leptodiscus* sp. are the most probable pathogens isolated.

A comparison of the suspect fungi obtained from unfavorable sites and favorable sites in the various fields is shown in Table 2 and indicates relatively little difference in the fungus flora or population. The unfavorable sites frequently involved heavier clay soils or poor water drainage and low populations of smaller plants. Usually these plants were more severely damaged by root-invading organisms. Thus it appears that the unfavorable environment in limited areas favored more extensive invasion by pathogens that were common throughout the area.

FUNGI ISOLATED FROM SEEDLING RED CLOVER ROOTS

Many more seeds are planted than can be accounted for by plants remaining in clover fields at the end of the first growing season. Frequently this reduction in numbers of plants can be correlated with the

TABLE 2

Frequency of Occurrence of Fungi Isolated from Clover Roots Growing in Favorable and Unfavorable Sites

	Unfavorable (Per Cent)	Favorable (Per Cent)
Fusarium oxysporum	24.3	23.9
Fusarium roseum	17.8	20.7
Fusarium solani	14.6	12.2
Gliocladium sp.	7.6	9.8
Rhizoctonia sp.	5.1	4.7
Phoma sp.	4.0	5.2
Fusarium moniliforme	2.98	2.4

appearance of visible root-rot symptoms and death of plants. It may appear in newly emerged seedlings as damping-off. This may not be visible without critical observations but it frequently involves large numbers of plants, especially during seasons when warm temperatures and high moisture occur during the germination period.

If large numbers of seedlings grow in the field, competition for space, nutrients and water become factors in reducing the vigor of some individuals. Larger plants usually persist and thrive. Smaller ones, frequently with distinct root-rot symptoms, make little growth and eventually die. The best evidence of the decrease in plant population is based on counts of plants made in measured areas over a period of time. Several dozen red clover seedlings as they emerge from the soil may occupy a square-foot area. By the end of the first growing season, perhaps one dozen or fewer plants survive in the same space. This conclusion is confirmed by plant counts made in Maryland by Allen and Kuhn (1).

The most significant feature of clover plant growth in this region is the apparent, nearly universal occurrence of *Fusarium* species in association with plants of all ages. *Fusarium* can usually be isolated from plants without visible root lesions of any kind. A lower incidence of *Fusarium* is found in seedling plants, but with increasing age, greater numbers of plants are contaminated. Only limited invasion of cells may occur until some later time when more extensive invasion may take place.

Fifty-plant random samples taken at intervals over a 12-week period beginning with seedlings revealed that roots of large numbers of plants had been contaminated with *Fusarium* species. In many instances, no visible lesion was observed in roots that yielded cultures of *Fusarium*. This was particularly true of the seedling material. Table 3 shows that in the area sampled, 16 per cent of the seedlings yielded *Fusarium* one week after planting seed, and by the time the plants were 12 weeks old, 94 per cent were contaminated.

TABLE 3

Numbers of Clover Plants Infected with Fusarium Species from 50-Plant Samples Taken at Various Intervals During the First Season of Growth

No. of Weeks After Planting	Species of Fusarium Isolated			Other	Total
	F. oxysporum	F. roseum	F. solani		
1	3	2	2	1	8
2	9	7	6	0	22
3	17	15	8	1	41
4	15	13	10	0	38
6	19	11	7	0	37
8	16	13	15	2	46
12	22	13	11	1	47

ASSOCIATION OF FUSARIUM WITH ROOT INJURIES

It is difficult to determine the origin of many of the injuries observed on the roots. The damage may be extensive such as that caused by the clover borer, *Hylastinus obscurus* Marsh. or the clover curculio, *Sitonia hispidula* F., (see Plate I) or may be very limited, such as that caused by feeding of ectoparasitic nematodes. Injuries may be from any sources but, no matter how large or small, each provides a point of entry for fungi that may extend into and invade uninjured cells.

Large populations of the clover root borer are found in all areas of the State where red clover is grown. The extensive tunneling of the larvae in the crown and tap root of two-year or older plants make an ideal site for growth and spread of the *Fusarium* which is generally associated with their feeding injuries. Extensive invasion by *Fusarium* and severe root borer injury are invariably found together.

The root surface wounds resulting from feeding of the clover curculio, S. *hispidula* are not as consistently contaminated by *Fusarium* spp., but nevertheless serve as a very frequent site of entry. The insect is abundant in West Virginia and the percentage of plants injured is high. At least some of the wounds ascribed to the feeding of *Sitonia* may be caused by other insects such as *Calomycterus setarius*. This weevil was reported from Pennsylvania by Newton and Graham (32), and the type of wounding it causes is much the same as that of the former insect. The present observations made in West Virginia fields of red clover follow the same pattern as the results obtained by Graham and Newton (19) in greenhouse tests. The most severe root rot developed in plants already invaded by the clover root borer, with less root rot in plants injured by the clover curculio alone.

NEMATODES ASSOCIATED WITH RED CLOVER ROOTS

Marked differences can usually be found in the numbers of red clover plants surviving during the second year of growth in wet locations at lower elevations of fields and those growing at more elevated points where better drainage usually exists. Low, wet locations invariably contain fewer surviving plants than the better drained sites. It was recognized that the soil particle size and organic matter content in these two site types within individual fields might have considerable effect on the different kinds and populations of nematodes.

The most common potentially pathogenic genera found were *Ditylenchus, Helicotylenchus, Hoplolaimus, Xiphinema, Pratylenchus,* and *Meloidogyne. Tylenchorhynchus* and *Paratylenchus* were also found in some numbers but are doubtful pathogens. In some instances, the population was considered high enough to be causing significant damage. More important though was the fact that a comparison of the numbers and kinds of nematodes from the low, wet locations and the high, well drained sites revealed no correlation between site and kinds or population of nematodes.

On the basis of this study made in 21 red clover fields in three counties, it appears that nematodes play no direct role in root rot, except that their feeding injuries in the root system likely provide a main avenue of entry for the several different fungi that are involved in root rotting. Undoubtedly various nematodes do reduce the vigor of these plants but no individual species was found associated with a major problem of red clover in the region.

OTHER DISEASES OF RED CLOVER

During the growing season one rarely finds individual plants that are free of foliage diseases. The severity of such diseases may vary widely but in many instances it is apparent they do reduce the overall vigor of plants. Black stem (*Phoma trifolii* Johnson and Valleau), *Stemphylium* leaf spot (*Stemphylium sarcinaeforme* Wiltshire), *Pseudopeziza* leaf spot (*Pseudopeziza trifolii* (Biv. Bern.) Fckl.), northern anthracnose (*Kabatiella caulivora* (Kirch.) Kavak.) and powdery mildew (*Erysiphe polygoni* D. C.) are widely distributed (see Plates I and II) and undoubtedly cause major reduction in forage quantity and value. None of these diseases are systematic in nature, and damage is generally in proportion to the number of infection points on the plant and virulence of the pathogen.

It is evident that in some instances these foliage diseases provide an additional stress on the plant where root rot is also present.

Red Clover Vein Mosaic Virus (RCVMV) is a widespread disease that few people recognize. It is most obvious during the cooler spring and fall seasons in leaves originating directly from the crown. The characteristic light and dark patterns of mosaic are illustrated in Plate I, illustration 4. The virus is present in all tissues of the plant. It causes a marked stunting and reduction in vigor, but alone is not lethal in red clover.

The combination of root rot and RCVMV is pronounced in some locations. At least a part of this relates to the age of the plants since both diseases are found with greater frequency as plant age increases. In some stands of red clover that are three years old, as high as 70 per cent of the plants are infected with RCVMV. The virus is aphid-transmitted and disease severity depends to some extent on the population of the vectors.

The association of root rot and RCVMV would indicate also that virus-weakened plants are more likely to be extensively invaded and killed by root rot fungi. Some field observations have indicated this. More critical greenhouse studies of this aspect are underway. Aphid control appears to offer an opportunity to increase yield and prolong the life of individual plants. Not only would aphid control help reduce the RCVMV, which is a major problem in its own right, but, also, if virus-weakened plants are more subject to invasion by root rot fungi, it would help reduce the root rot problem and prolong the life of the stand.

Alfalfa Diseases

Many diseases of alfalfa are confined mainly to the root system or to the root and crown. The pathogens causing these diseases are generally thought of as being intimately associated with the soil. Specifically the soil provides a favorable protective environment for these disease organisms during much of their life.

SCLEROTINIA ROOT AND CROWN ROT

Little needs to be added to a description of *Sclerotinia* rot that appears in the section on red clover diseases. The same fungus, *Sclerotinia trifoliorum* Erikss., attacks both alfalfa and red clover and several other forage legumes. In the West Virginia area, *Sclerotinia* is usually less severe in alfalfa plantings than in red clover. Diseased and dying alfalfa plants are evident about 2 weeks later than diseased red clover plants. This does not mean that infection has occurred later but perhaps only that the fungus takes longer to invade the plant and cause readily observed injury.

None of our present alfalfa varieties are resistant to this disease. *Sclerotinia* is particularly difficult to control because of the apparent lack of resistance that can be utilized by plant breeders.

BACTERIAL WILT

The most widely destructive alfalfa disease in the United States is caused by *Corynebacterium insidiosum* (Mc Cull.) H. L. Jens. This disease was not described until 1925 when the casual bacterium was found, but it was probably widespread in alfalfa-growing regions before that time (24).

Bacterial wilt is mainly confined to the vascular tissues. The tap-root and crown serve as a reservoir from which the bacteria spread into new branches as they are produced. Invasion by the bacteria results in dwarfed, yellowed foliage, with small, narrow leaflets that are curled at their margins. Wilting is frequently observed during periods of high temperature. Vascular tissues are yellow or brown when the taproot is cut open.

The bacteria released from decayed plants during the spring and early summer may enter uninfected alfala through wounds of various kinds. Infected plants usually die during the summer of the second year.

When bacterial wilt was described and found in many of the alfalfa-growing regions in the United States, it was frequently assumed that any cases of dying plants and poor stands were a result of the disease. This often occurs when a disease receives widespread publicity as did alfalfa wilt. The growers were led to believe that the disease was present in each area and that bacterial wilt-resistant varieties, when they became available, would solve the poor stand problems.

As with *Sclerotinia* crown rot of legumes, it is difficult to trace the true history of alfalfa wilt in West Virginia. Archer (9) did not report the disease in 1928 nor did Friant (16) in 1945. Atkinson *et al.* (4) found the disease in Northern Panhandle hillside fields in 1945 and reported that it was not observed in other parts of the State.

More recent observations indicate scattered occurrence in the Eastern Panhandle, Northern Panhandle, and south along the Ohio River, but the disease is uncommon. On the basis of yield and survival of plants, the varieties Vernal, Narragansett, and Williamsburg are the most recently recommended for long-term stands. Since these varieties have been tested widely in the State, this is additional evidence of the minor nature of alfalfa wilt in the region; both Narragansett and Williamsburg are susceptible.

FUSARIUM WILT

Weimer (44) reported a Fusarium-wilt of alfalfa producing symptoms similar to those resulting from bacterial wilt. In a later report (45), the fungus was identified as *Fusarium oxysporum* and was described as a new variety (*F. oxysporum* var. *medicaginis*).

This typical Fusarium-wilt alfalfa is caused by a highly virulent fungus that occurs in several parts of the United States but it has not been specifically identified from West Virginia. Additional comments on this wilt disease appear in the next section on root rots.

FUSARIUM ROOT ROTS

Fusarium species are involved in a variety of alfalfa problems in North America. Information on Fusarium range from reports of the highly virulent pathogens such as the alfalfa wilt Fusarium described by Weimer (45) and the Fusarium dry root rot disease found by Cottam (12) in Utah to the reports of Busch and Gilpatrick (7) that *Fusarium* species isolated from alfalfa and sweetclover were probably secondary or weak parasites.

Several of the reports on the pathogenicity of *Fusarium* to red clover, alfalfa and other forage legumes have been based on observations. Some workers have reproduced or have attempted to reproduce the disease using the various species or isolates they have had available. Here again the reports have varied widely. Weimer (45) readily obtained diseased plants in the greenhouse when he inoculated alfalfa with *F. oxysporum* var. *medicaginis*. Staten and Layendecker (39) found *Fusarium solani* isolates from New Mexico induced typical root rotting of alfalfa in greenhouse plants. Many investigators have found that a wide spectrum of strains may exist, varying from highly virulent to weakly virulent, within a single *Fusarium* species.

The present investigation reveals that *F. oxysporum, F. roseum* and *F. solani* are the species most commonly isolated and that there is a wide range of virulence among the different isolates. Table 4 records the frequency of occurrence of fungi isolated from four-year-old DuPuits alfalfa during a one-year period. This is typical of the variety of fungi isolated at any location. When numbers and kinds of fungi isolated from vigorous and unthrifty plants are compared, surprisingly little difference is noted indicating the common association of plant and potential pathogen.

These fungi are associated with the surface and interior of the root system of perennial plants, and one finds relatively little change in this flora over a period of time. Essentially the same fungi are recovered in the winter as are found in the summer.

PLATE I. 1—Longitudinal section of red clover root revealing Fusarium root rot, a tunnel of the clover root borer and possible internal breakdown (IB) in the upper crown. 2—Typical curculio feeding injury on red clover roots. 3—Curculio feeding injury and associated Fusarium invasion of red clover roots. 4—Red clover vein mosaic virus (RCVMV). 5—Blackstem of red clover. 6—Stemphylium leafspot of red clover.

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PLATE II. 1—Serial sections of alfalfa roots showing invasion by Fusarium sp. 2—Curculio feeding injury on alfalfa roots. 3—Blackstem of alfalfa. 4—Alfalfa weevil. 5—Pseudopeziza leafspot of red clover. 6—Red clover powdery mildew.

TABLE 4

Fungi Isolated from Vigorous and Unthrifty Four-Year-Old DuPuits Alfalfa Plants Over a One-Year Period

Organism	Vigorous Plants (% of Total)	Unthrifty Plants (% of Total)
Fusarium oxysporum	30.7	54.8
F. roseum	15.5	15.4
F. solani	9.8	12.2
F. moniliforme	22.0	9.6
Leptodiscus terrestris	8.0	3.4
Rhizoctonia sp.	1.5	0.8
Other fungi*	12.5	3.8

*Species of *Alternaria, Gliocladium, Rhizopus, Trichoderma, Mucor, Aspergillus, Penicillium, Epicoccum,* and *Botrytis.*

NEMATODES ASSOCIATED WITH ALFALFA ROOTS

A general survey of nematodes associated with alfalfa plants has not been made for the region, but data from two locations provide some evidence of the kinds that are present. The numbers and types of nematodes collected and identified from one of these locations are presented in Table 7 (page 23). The samples were obtained from roots and surrounding soil collected in a field of four-year-old DuPuits alfalfa. Samples were taken at monthly intervals from March to August. On the basis of general appearance of the plant, half the samples were obtained from vigorous plants and the remainder from unthrifty plants.

Table 7 shows that more nematodes, including a greater percentage of nonparasitic forms, were extracted from soil surrounding the roots of vigorous plants. More parasitic nematodes were extracted from soils surrounding the roots of unthrifty plants and it should be noted that in this classification some of the genera that contain species parasitic on alfalfa had to be placed under "questionable parasitism" because none of the nematodes were identified to species.

Free-living forms predominated in the soil surrounding both unthrifty and vigorous plants. The genera thought to be potential alfalfa feeders, listed according to the frequency of their occurrence, includes *Aphelenchus* (most common), *Hoplolaimus, Aphenlenchoides, Pratylenchus, Xiphenema, Criconemoides,* and *Ditylenchus* (least common). Larvae of nematodes of the family Heteroderidae were found in limited numbers.

Table 7 also shows that approximately twice as many nematodes, including a greater percentage of parasitic forms, were extracted from incubated roots of unthrifty plants than from roots of vigorous plants. The

list of probable alfalfa-feeding kinds from incubated roots is similar but not the same as from the surrounding soil. *Aphelenchus* is again the most common genus, followed by *Xiphenema, Trichodorus, Hoplolaimus, Aphelenchoides, Pratylenchus* and *Ditylenchus*. Ectoparasitic forms extracted from the incubated roots may have been included in decayed areas or in crevices of the roots when they were washed before incubation.

In both sampling techniques used, greater numbers of parasitic genera were found associated with unthrifty plants. At this location, and this may be applicable to some similar situations, nematode feeding appeared to be reducing the vigor of the plants. The extent of this reduction could not be measured because other factors, such as more extensive invasion of unthrifty plants by *Fusarium* species, were involved. Also unmeasured was the importance of nematode-feeding injuries as entry points for the spectrum of parasitic fungi. These areas of injured cells also provide a site for at least limited growth of microorganisms that are not parasitic which may have adverse effects on adjoining living tissues of the plant.

Another area of one-year-old DuPuits alfalfa was sampled at monthly intervals for 10 months and a similar group of nematodes was found associated with the plants. Free-living forms made up approximately 70 per cent of the population. The genera *Aphelenchus, Aphelenchoides, Ditylenchus, Hoplolaimus, Pratylenchus* and larvae from the family Heteroderidae are possible alfalfa feeders. Even though the amount of injury to alfalfa by these nematodes is unknown, this provides additional evidence of their association.

OTHER DISEASES OF ALFALFA

Severe damage to the alfalfa crop may also result from epiphytotics of certain foliage diseases. No attempt will be made to describe these diseases in detail but only to point out those that are major problems in the region. These have been listed and described for the Northeastern States by Kreitlow, Graham and Garber (27). This information is applicable to West Virginia.

The lower and older leaves of the alfalfa plant are more subject to infection by pathogens because of longer exposure to greater quantities of free-water as rain or dew and to continuing high levels of moisture in the air. This moist microclimate found beneath the upper canopy of leaves is a very favorable environment for fungus pathogens. A characteristic of alfalfa is that leaves or leaflets frequently drop to the ground within a few days after infection has occurred. This defoliation resulting from leaflet infection by any one of several alfalfa diseases is usually the

most evident injury, but frequently the grower does not recognize the cause of defoliation. The result is a flowering alfalfa plant at the proper stage for cutting composed mainly of stems with few leaves.

Black stem (*Ascochyta imperfecta*) is the most widespread foliage disease observed in the spring (see Plate II, illustration 3). The fungus overwinters in the dead stems and produces spores in the spring and early summer that are splashed to the new growth. Irregular brown to black lesions appear on leaves and stems and cause some defoliation. The yield and quality of forage is reduced in proportion to the severity of the disease.

Common leaf spot or Pseudopeziza leaf spot (*Pseudopeziza medicaginis*) is the most prevalent disease found during the warmer summer months in West Virginia. Small circular lesions on the leaflets are followed by general yellowing and leaf drop. A prominent, raised fruiting body in the center of the small leaf spot is the best means of identifying this disease. Defoliation is confined mainly to the second and third hay cuttings.

A third common disease causing defoliation in the region is called Pseudoplea leaf spot (*Pseudoplea trifolii*). The lesions are small and resemble common leaf spot but no raised fruiting body is present. Yellow leaf blotch (*Pseudopeziza jonesii*), summer black stem (*Cercospora zebrina*) and downy mildew (*Peronospora trifoliorum*) are less common, though they also contribute to the defoliation problem.

The defoliation aspect has been emphasized in these descriptions because it not only affects the quantity and quality of the harvested forage but also contributes to a reduction of plant vigor. Foliage-feeding insects, particularly the alfalfa weevil, add to this stress. O'Rourke and Millar (33) have shown in greenhouse tests that the amount of Fusarium root rot increased with increased levels of foliar infection when the plants were inoculated with *Ascochyta imperfecta*. Plants with reduced vigor resulting from foliar diseases are undoubtely more susceptible to root rot invasion.

Pathogenicity of Fungi Isolated from Alfalfa and Red Clover Roots

Numerous studies have been made to determine the pathogenicity or relative virulence and importance of different species of fungi in the development of root rots of both alfalfa and clover. These have utilized several different techniques and employed plants of various ages grown under different conditions. Many of these tests have been made with

seedling plants. Crall (13) found that isolates of *F. oxysporum, F. solani, F. moniliforme,* and *F. roseum* from first-year red clover in Iowa exhibited much variation in reducing seedling stands of red clover in artificially infested greenhouse soils. Numbers of surviving seedlings at the end of one month were used as a measure of virulence by Ostazeski and Gerdemann (34) in determining the effects of different methods of soil infestation on the pathogenicity of *F. solani, F. oxysporum* and *Gliocladium roseum* from red clover roots. Fulton and Hanson (17) demonstrated a wide variation in virulence in fungi isolated from red clover roots and tested on plants grown in white silica sand, fumigated soil, steamed soil, and naturally infested soil. Other similar studies on root fungi isolated from clover and alfalfa have been reported by McVey and Gerdemann (29), Chi and Hanson (10), and McCarter and Halpin (28).

Many of these studies have recorded that some species of fungi isolated from roots of these plants are capable of killing seedlings or of invading the root systems of more mature plants and inducing disease. These studies also indicate a wide range of virulence among the isolates of the same species.

Using a group of fungus isolates obtained from rotting roots of red clover and alfalfa, an attempt was made to determine the relative virulence of individual isolates to seedlings and to 6-month-old plants of alfalfa and red clover. Seedling damping-off can be obtained much more rapidly than the typical root rotting of older roots and thus might serve as a quick assay of isolate virulence if it represents similar potential for mature root invasion. Table 5 shows the damage that resulted when a group of isolates from alfalfa roots was tested on seedlings and 6-month-old red clover and alfalfa plants. The data in Table 6 are similar, using isolates obtained from red clover roots. Seedlings grown in sterilized white silica sand in 400-ml beakers capped with petri dish bottoms were used in damping-off tests. A nutrient solution was added to the sand before the surface-sterilized seeds were planted and incubated at 30° C. The number of dead seedlings were counted at weekly intervals; only the counts made at the end of 2 weeks are included in Table 5 and 6.

Six-month-old greenhousegrown plants from methyl bromide-sterilized soil were inoculated by removing the plant from the soil, washing in tap water, cutting away the bottom one-fourth of the root length and dipping the remaining roots in an inoculum mixture. These plants were then repotted in fumigated soil and held in the greenhouse at temperatures ranging usually between 80 and 85° for another six months.

A scale rating the various kinds and degrees of injuries permitted an assay of total damage to the 1-year-old plants. Tables 5 and 6 show that some contamination occurred in the pots of control plants. The omni-

TABLE 5

Virulence of Fungi Isolated from Alfalfa Roots and Tested on Seedling and Mature Penn Scott Red Clover and DuPuits Alfalfa Plants

		Per Cent Damage to Red Clover		Per Cent Damage to Alfalfa	
Fungus	Isolate Number	Dead Seedlings*	Mature Roots**	Dead Seedlings	Mature Roots
Fusarium roseum	61	100	78	100	53
F. moniliforme	2B	100	75	100	40
	22A	95	68	95	45
F. solani	73A	100	88	100	50
	62	92	76	97	80
	12B	99	83	100	38
F. oxysporum	76B	100	81	99	50
	37C	98	93	100	50
Leptodiscus terrestris	W3A	100	90	100	55
Control		0	23	0	15

*Two weeks after inoculation.
**Six months after inoculation.

TABLE 6

Virulence of Fungi Isolated from Red Clover Roots and Tested on Seedling and Mature Pennscott Red Clover and DuPuits Alfalfa Plants

		Per Cent Damage to Red Clover		Per Cent Damage to Alfalfa	
Fungus	Isolate Number	Dead Seedlings*	Mature Roots**	Dead Seedlings	Mature Roots
Fusarium roseum	54A	100	80	100	43
	132	98	95	100	45
	125	79	60	96	45
F. moniliforme	95	100	80	100	80
	93	94	78	90	45
	120	78	61	40	41
F. solani	98	90	78	100	65
	127	96	80	86	50
	131	98	88	85	48
F. oxysporum	68DA	86	95	94	68
	68DB	77	95	84	53
	150A	77	75	43	51
Control		0	23	0	15

*Two weeks after inoculation.
**Six months after inoculation.

TABLE 7
Number and Types of Nematodes Extracted from Roots of Unthrifty and Vigorous Four-Year-old DuPuits Alfalfa

Source of Sample	Total Number Extracted		% Parasitic		% Nonparasitic Forms		% Questionable Parasitism		Number of Nematodes in Sample	
	U*	V*	U	V	U	V	U	V	U	V
Extracted from soil around the roots	1.715	2,630	11.1	6.4	56.9	62.8	32.0	30.7	250	325
Extracted from incubated roots	852	466	10.2	2.8	79.4	76.9	10.4	20.3	267	246

*U = Unthrifty; V = Vigorous.

present Fusaria are very difficult to keep in place even in the greenhouse. The contaminants may have been present in the roots at the time of inoculation or may have been airborne material that resulted in infection at a later time. Usually, the same species was obtained on re-isolation from the infected plants but occasional contaminants were also detected in this way.

The greenhouse temperature was maintained at a relatively high level (80-85° F) since this range has been found to be optimum for disease development. DuPuits alfalfa is relatively susceptible to root rot injury, thus other varieties might show less damage if subjected to the same fungus isolates.

Fusarium root rot injury of alfalfa is illustrated in Plate II, illustration 1.

Leptodiscus terrestris, a fungus described by Gerdemann (18) as causing a root rot of red clover and other legumes in Illinois, is occasionally isolated from both alfalfa and red clover in this region. Table 5 shows that damage resulting from infection by this pathogen is equal to that caused by any of the *Fusarium* isolates, but due to its present limited distribution in this region, it does not now present a serious problem.

A significant result of this study is the evidence that individual isolates readily attack both hosts regardless of whether they came initially from red clover or alfalfa. Usually less injury was evident in alfalfa than in red clover. This agrees with field observations in the region and in other areas of the United States. Although these specific isolates have not been tested it seems reasonable to believe that they are potentially pathogenic on other legumes, on grasses, including cereals and on other crop plants.

Root Rots Associated With Injuries

Insect injury to the roots of alfalfa appears to be largely that of the root curculios *Sitonia flavescens* Marsh and S. *hispidula* Fab. (see Plate II, illustration 2). No critical study of these insects has been made on alfalfa but an earlier report (14) indicates that S. *flavescens* is the most common species associated with red clover in the region. The feeding injuries of these insects, mainly confined to the root cortex, provide ideal sites for entry of soil fungi. No study has been made to determine the frequency of entry through such wounds except to note that roots showing large numbers of feeding scars are more likely to be extensively invaded by a Fusarium.

The clover root borer (*Hylastinus obscurus* Marsh.) has rarely been observed to injure alfalfa. The extensive injuries to the tap root providing

an ideal situation for invasion, is definitely one of the keys to the greater losses sustained by red clover from Fusarium root rot. No similar widespread severe injuries can be found in alfalfa.

Insect feeding on the aerial parts of the plant would reduce plant vigor and potentially could contribute to root-rot problems. The extensive feeding damage of the alfalfa weevil, *Hypera postica* (Gyll.) would be suspect in this region (Plate II, illustration 4.) and also the widespread reduction in plant growth caused by potato leafhopper [*Empoasca fabae* (Harris)] feeding injury.

Injuries occurring during the winter months are difficult to assess and they are particularly difficult to relate to entry and spread of pathogenic fungi. Frost heaving resulting in lifting of the tap root and breaking of lateral roots is an extreme type of cold injury and whether or not the roots are invaded is of little importance since the mechanical injury is often sufficient to kill the plant. The many smaller injuries that result from freezing and thawing provide sites through which pathogens may invade. The injury is not confined to the root system since the succulent top growth is quickly frozen back to the crown area leaving unprotected stubs of dead tissue as suitable sites of entry.

A Summary of the Fusarium Root-Rot Problem

The information from the present study and published reports contributes to a better understanding of Fusarium root rot of clover and alfalfa. To provide a background for the following section on control, we summarize what is known about the disease from scientific facts, practical experience, and circumstantial evidence.

1. The origin of *Fusarium* species that attack these legumes is obscure. They may have spread with the movement of the crop into new areas or they may have been a normal component of the soil microflora in most areas. The latter premise seems more logical because of the known characteristics of the genus *Fusarium*. This cosmopolitan group is composed of highly variable species. They appear to adapt, in many instances, to a low degree of parasitism on many seed plants and only a few reach a point where they are highly virulent to specific plant species. Alfalfa and red clover are particularly suitable hosts. Obviously pathogenic types tend to build up in an area where a favorable host is present.

2. Red clover or alfalfa seeded on cultivated soil are surrounded by large numbers of potentially pathogenic *Fusarium* spp. Large numbers of

seedlings may be killed (damping-off) before or after they emerge from the soil, particularly during periods of warm, wet weather.

3. Species of *Fusarium* enter the roots of young plants by direct penetration (11) or through wounds caused by feeding of nematodes, or insects, or by other agents. Depending on its virulence, the fungus may continue to advance in the tissues of the plant or, in many instances, growth is limited to small areas of wounded- or normal-surface cells. From this initial area of invasion, it may extend into the vascular tissues at some later time when plant vigor is reduced. The best evidence for this is that virulent isolates can be obtained from the outer root tissues of all ages of plants. These plants show no evidence of invasion of the vascular tissues.

4. As the plants increase in size, competition for space becomes a limiting factor. The greater the numbers of surviving seedling plants, the more critical this becomes. Clover plants and weeds (1) because of suitable nutrition or other factors favoring rapid growth become dominant. Because of this competition, nearby plants become increasingly less vigorous. These weakened plants may die during the early weeks or months of crop growth. In many instances the roots have been invaded or destroyed by *Fusarium*.

5. Low temperatures and periodic freezing and thawing adversely affect the dormant overwintering plants. Marked differences exist in the winter-hardiness of both alfalfa and red clover varieties. Alfalfa varieties exposed to extreme winter conditions, particularly the less hardy types, are vulnerable to winter injury and, at the same time, to invasion by *Fusarium* spp. Extensive Fusarium root rot is often found in plants lifted by frost heaving.

6. The stage of plant growth, number of harvests, and timing of harvest are all factors that may adversely affect plant vigor. O'Rourke and Millar (33) found that increased frequency of cutting of alfalfa increased the incidence of Fusarium root rot. Fulton and Hanson (17) reported the same to be true for red clover. Cutting alfalfa late in the growing season, thereby reducing stored food reserves before winter dormancy, has long been recognized as a poor practice.

7. Drought and associated high temperatures most frequently occur during the summer months and provide a serious stress, particularly in red clover. Dying plants, examined during or following lengthy periods of drought, show a high incidence of root rot. Red clover in West Virginia frequently fails to recover after the first cutting of the second-year's growth when high temperatures and drought occur during this period. The clover root borer injury is also most extensive during this first cutting

period. This combination of cutting stress, drought, insect injury, and root rot invasion is devastating and the plants die in great numbers.

Although no studies have been made in this region on the effects of excess water on plant survival, it is evident that fewer plants remain during the second year in poorly-drained areas than in well-drained soils. This may also be related to winter injury, particularly to frost heaving in wet locations. Dying plants from these areas also are usually invaded by *Fusarium* species.

8. Insect injuries of various kinds have an adverse influence on plant vigor. Alfalfa weevil feeding on alfalfa plants or root borer damage to red clover roots alone can be sufficient to kill the plants. Usually insect injury is less extensive but the combined feeding of many kinds of insects results in weakened plants. Graham and Newton (19) have shown that insect injury is intimately related to Fusarium root rot of red clover.

9. Many studies have been made on the effect of plant nutrition as it relates to longevity of these legumes but these studies have not included the relationship of plant nutrition to the Fusarium root rot problem. Chi and Hanson (10) demonstrated that more red clover rot developed at low nutrient concentrations than at optimum or above optimum concentrations for plant growth. Stivers *et al.* (40) correlated low fertilization rates with increased occurrence of root rot in alfalfa field plots, but they could not prove that the isolates of *F. oxysporum, F. roseum,* and *F. solani* obtained from the roots were pathogenic. O'Rourke and Millar (33) found that additional potassium fertilization effectively reduced the losses from Fusarium root rot in alfalfa field plot trials.

10. The reduction in plant vigor associated with diseases other than Fusarium root rot also may have a marked effect on the severity of root rot. O'Rourke and Millar (33) demonstrated this with plants inoculated with the black stem fungus (*Ascochyta imperfecta*). The early loss of alfalfa leaves because of leaf infecting fungi is a good example of this problem.

Controlling Fusarium Root Rots

The summary of the many factors that are associated with Fusarium root rot of red clover and alfalfa is good evidence of the complex interrelated nature of the problem. It is evident also that control of the disease is complicated. Most of the practices recommended for red clover and alfalfa culture are related in some way to control of root rot because these are aimed at producing a vigorous, high-yielding, long-lasting crop. But additional methods are needed to further reduce the loss of these crops.

Two general approaches to the control problem are evident—the first is to control or manipulate the various factors that may increase root rot and the second is to produce varieties of forage crops resistant to the several *Fusarium* species involved.

RESISTANT VARIETIES

Many of the newer alfalfa varieties have resistance to certain diseases and insect pests but few have resistance to Fusarium root rot. The variety Teton is a good example of the progress made by plant breeders in obtaining disease resistant material. Hanson *et al.* (21) report that Teton is resistant to bacterial wilt, common leaf spot and field infection caused by *F. oxysporum* and *F. roseum*. In the registration description (22) this variety is also described as resistant to Phoma blackstem, yellow leaf blotch and alfalfa rust, but susceptible to Cercospora and Pseudoplea leaf spots. Teton was developed by the South Dakota Agricultural Experiment Station and, understandably, is better adapted to conditions of the Northern Great Plains region.

In New Mexico, Wilson and Melton (46) developed synthetic varieties with more resistance to *F. solani* than was found in New Mexico Common. The variety Zia from that station has resistance to the spotted alfalfa aphid and more resistance to bacterial and Fusarium wilts than New Mexico Common.

These examples of specific resistance to some *Fusarium* spp. serve as an indication that varieties adapted to certain regions with even higher levels of resistance to root rots can be obtained. The knowledge that a number of *Fusarium* species are usually involved in the root-rot complex in most areas does not simplify the problem, neither does it make it insurmountable.

Certain selections of red clover available through the Department of Agriculture plant introduction stations have been designated as having Fusarium root rot resistance but this has not been incorporated into any of the existing available varieties. Since red clover is more susceptible to the root-invading fungi, the task of producing acceptable root rot resistant material of this legume will probably be more difficult than that of alfalfa. Seed of locally produced strains of red clover that have adapted to a certain area through natural selection may be the best source of root rot resistant plants. When comparisons of plants are made within a specific area, the local strains grown for many generations frequently out yield and live longer than improved varieties or strains from other areas.

The increasing extent of foliage disease resistance that is being incorporated into alfalfa varieties and, to a lesser extent, into red clover

varieties is an indirect means of reducing root rot losses. A similar means is provided by varieties of legumes resistant to insect injury that help to retain vigorous plants less susceptible to root rot injury. Resistance to certain virus diseases is another means of improving the vigor of these plants in the future.

The varieties of these forage crops that are highest yielding and best adapted to West Virginia conditions contain either some resistance to the organisms involved in this complex or they yield better even with an average level of root rot present. The recommended varieties should be used until better varieties, adapted to the region, are found.

CONTROL OF INSECTS

In order to fully utilize alfalfa as a forage in West Virginia it has been necessary in recent years to apply insecticides to control the alfalfa weevil. Previous to the time this insect spread across the State, insecticides were used primarily to reduce losses from feeding of leafhoppers and spittlebugs. Efforts to control these insects have been hampered by restrictions placed on the use of certain insecticides, but other acceptable but less effective materials are widely used. Usually no effort has been made to control red clover insect pests.

At least two things that relate to root rot control are accomplished with the alfalfa spray program; the plant vigor is maintained at a higher level and at least some reduction is obtained in root feeding by certain insects. Some control of aphids that spread the alfalfa mosais virus is also obtained.

In the central Appalachian region the clover root borer and root curculios cause severe injury to the roots of red clover. If these insects could be controlled with acceptable insecticides, it would reduce the direct loss from insect feeding and provide the quickest means of obtaining major reductions in root rot losses. As in alfalfa, adequate aphid control should reduce the spread and resulting losses from virus diseases, particularly the red clover vein mosaic virus.

MANAGEMENT PRACTICES

Management practices as a means of root rot control can be summarized by the statement that all practices resulting in fast growing, vigorous plants are useful. Adequate nutrients and proper timing of cutting are two important considerations. Except for irrigation, little can be done about periods of drought other than the selection of planting site. Usually the shallow upland soils are subject to more extreme drought problems than the deeper soils found in valleys.

Seed treatment with chemicals appears to offer a means of reducing the loss of seedlings from damping-off by *Fusarium* species and certain other fungi. There are conflicting reports (2, 23, 26) on the value of chemical seed treatment, which perhaps is the reason that the practice is not generally recommended.

Crop rotation is frequently suggested as a means of reducing losses from disease and is applicable in instances where the quantity of inoculum is reduced by keeping the susceptible host plant away for an extended period of time. Due to the ability of *Fusarium* species to parasitize a variety of different plant species or to exist as saprophytes in the soil, rotation is of little value as a practical means of reducing populations of these root rot fungi.

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